



# Taxonomy of the Colocasiomyia gigantea species group (Diptera, Drosophilidae), with descriptions of four new species from Yunnan, China

Nan-Nan Li<sup>1,2,†</sup>, Masanori J. Toda<sup>3,‡</sup>, Zhao Fu<sup>1,§</sup>, Ji-Min Chen<sup>1,†</sup>, Su-Hua Li<sup>2,¶</sup>, Jian-Jun Gao<sup>1,#</sup>

Laboratory for Conservation and Utilization of Bioresources, Yunnan University, Kunming, Yunnan 650091, China 2 School of Forestry, Southwest Forestry University, Kunming, Yunnan 650224, China 3 Hokkaido University Museum, Hokkaido University, Sapporo, Japan

- † http://zoobank.org/3EC95EBB-9DDB-4BF7-A346-9AE3DF2A1C9B
- ‡ http://zoobank.org/C8298109-8044-4317-BC36-69367954AC6F
- § http://zoobank.org/12CC1FCF-A443-4486-8B46-22784FE01470 | http://zoobank.org/705F5D95-EE6E-4426-9247-B8AB6EC5F240
- ¶ http://zoobank.org/967C6BE0-8CFB-40D0-9B9C-50419D47C037
- # http://zoobank.org/082D071C-E61E-4394-BFBA-0D876F4BA4FC

Corresponding author: Jian-Jun Gao (gaojj@ynu.edu.cn)

Academic editor: R. Meier | Received 1 February 2014 | Accepted 16 April 2014 | Published 30 April 2014 |

http://zoobank.org/AF68F25C-57E0-4633-94D0-9E566D97A951

**Citation:** Li N-N, Toda MJ, Fu Z, Chen J-M, Li S-H, Gao J-J (2014) Taxonomy of the *Colocasiomyia gigantea* species group (Diptera, Drosophilidae), with descriptions of four new species from Yunnan, China. ZooKeys 406: 41–64. doi: 10.3897/zookeys.406.7176

## Abstract

Species of the genus *Colocasiomyia* de Meijere feed/breed on inflorescences/infructescences of the plants from the families Araceae, Arecaceae and Magnoliaceae. Although most of them utilize plants from the subfamily Aroideae of Araceae, three species of the recently established *C. gigantea* species group make use of plants of the subfamily Monsteroideae. We describe four new species of the *gigantea* group found from Yunnan, China: *Colocasiomyia longifilamentata* Li & Gao, **sp. n.**, *C. longivalva* Li & Gao, **sp. n.**, *C. hailini* Li & Gao, **sp. n.**, and *C. yini* Li & Gao, **sp. n.** The species delimitation is proved in virtue of not only morphology but also DNA barcodes, i.e., sequences of the partial mitochondrial *COI* (cytochrome *c* oxidase subunit I) gene. Some nucleotide sites with fixed status in the alignment of the *COI* sequences (658 sites in length) are used as "pure" molecular diagnostic characters to delineate species in the *gigantea* group.

#### **Keywords**

Adaptation, aroid, character-based barcoding, cohabitation, genetic distance, integrated taxonomy, *Rhaphidophora* clade

#### Introduction

To date, as many as 90 species (of them only 25 species described) have been found in the genus *Colocasiomyia* de Meijere, 1914. All these species visit and breed on flowers of the families Araceae, Arecaceae or Magnoliaceae (Sultana et al. 2006, Fartyal et al. 2013, Toda unpublished data). So far, six species groups, i.e., *baechlii*, *cristata*, *toshiokai*, *crassipes*, *zeylanica* and *gigantea* (Okada 1990, Sultana et al. 2002, 2006, Fartyal et al. 2013), have been established in this genus.

The gigantea group was recently erected by Fartyal et al. (2013) for two species [C. gigantea (Okada, 1987) and C. scindapsae Fartyal & Toda, 2013] from Southeast Asia and one species (C. rhaphidophorae Gao & Toda, 2013) from China (Table 1). Host plants of these species belong to the Rhaphidophora clade of the subfamily Monsteroideae of True Araceae, according to the most recent phylogenetic studies of the Araceae (Cusimano et al. 2011, Nauheimer et al. 2012). On the other hand, the other species groups utilize either the subfamily Aroideae or the other families. Fartyal et al. (2013) investigated the reproductive ecology of the three species, and revealed interesting ecological and morphological adaptations of the flies to the host plants. In addition, Fartyal et al. (2013) conducted a cladistic analysis of 70 morphological characters of 34 Colocasiomyia species, covering all the six species groups of the genus. Their results lent essential support to the monophyly of the gigantea group, placing it as the sister clade to the cristata species group.

**Table 1.** Summary of the species of the *Colocasiomyia gigantea* species group.

Species name	Distribution	Host plant	Reference	
C. gigantea (Okada, 1987)	Java, Indonesia; Solomon Is.	Epipremnum pinnatum (L.) Engle	Fartyal et al. (2013)	
C. scindapsae Fartyal & Toda, 2013	Sabah, Malaysia	Scindapsus coriaceus Engler	Ditto	
Caladilahan Carettal	Xishuangbanna, Yunnan, China	Rhaphidophora hookeri Schott	Ditto	
C. rhaphidophorae Gao & Toda, 2013	Pu'er, Yunnan, China	Rhaphidophora decursiva (Roxb.) Schott	Present study	
C. longifilamentata sp. n.	Baoshan and Pu'er, Yunnan, China	Ditto	Ditto	
C. longivalva sp. n.	Ditto	Ditto <sup>a</sup>	Ditto	
C. hailini sp. n.	Ditto	Ditto	Ditto	
C. yini sp. n.	Ditto	Ditto	Ditto	

<sup>&</sup>lt;sup>a</sup> To be confirmed by further investigation (for details, see the "Remarks" section in the description of *C. longivalva* sp. n.)

Our recent field surveys in Yunnan Province, China brought new, insightful information on the evolution of flower-breeding habits in the *gigantea* group. We found four new species of this group visiting inflorescences of *Rhaphidophora decursiva* (Roxb.) Schott (Table 1); at least three of them were found breeding on inflorescences/infructescences of this plant. In Pu'er (central-southern part of Yunnan), *C. rhaphidophorae* cohabited with the above-mentioned three new species on inflorescences/infructescences of *R. decursiva*. Thus, the Chinese members of the *gigantea* group are mostly sympatric and overlapping in host plant selection with each other (cohabitation), in contrast to the allopatry and monopolization of host plant in the other members, *C. gigantea* and *C. scindapsae*.

The four new species of the *gigantea* species group are described here, based on species delimitation in virtue of morphological and molecular (DNA sequences of the mitochondrial cytochrome *c* oxidase subunit I gene, *COI*, as the DNA barcoding marker) characters.

#### Materials and methods

#### Materials

Table 2 shows the fly samples/specimens involved in the present study. Most of them were collected from the field in southwestern China, Malaysia and Indonesia. Some were reared from inflorescences/infructescences of host plants; after dissection of inflorescences/infructescences under a stereoscopic microscope in laboratory, the fly eggs were isolated and transferred into Petri dishes with decayed pistils as food and then reared at 25°C in an incubator until adults emerged.

## Morphological observation

We followed the same method as in Fartyal et al. (2013) for the observation of external morphology, measurement of morphometric characters and preparation of dissected organs. The male and female terminalia and cibarium of the new species were microphotographed using a DinoLite Digital Eyepiece Camera and drawn according to these digital pictures using CORELDRAW® X4 (Corel Corporation). Fine structures of foreleg (tibia, 1st and 2nd tarsomeres) and oviscapt were microphotographed for all the seven species of the *gigantea* group using a HITACHI TM3000 tabletop scanning electron microscope (SEM). We followed McAlpine (1981) for the morphological terminology, and Zhang and Toda (1992) for the definitions of measurements and indices. New type specimens were deposited in Kunming Natural History Museum of Zoology, Kunming Institute of Zoology, Chinese Academy of Sciences (KIZ) and Hokkaido University Museum, Hokkaido University, Sapporo, Japan (SEHU).

Species	Voucher #/GenBankaccession number	Collection site <sup>d</sup>
C. sulawesiana	Lot055 (DNA)/KJ700880 <sup>a</sup>	A
C. colocasiae	Lot072 (DNA)/KJ700879 <sup>a</sup>	В
C. xenalocasiae	001627/KJ700881	С
C. gigantea	Lot150 (DNA)/KJ700882 <sup>a</sup> ; 001444/KJ700883 <sup>a</sup> ; 001445/KJ700884 <sup>a</sup> ; 001449/KJ700885 <sup>a</sup>	D
C. scindapsae	Lot036 (DNA)/KJ700886 <sup>a</sup> ; Lot037 (DNA)/KJ700887 <sup>a</sup> ; 001186/ KJ700888 <sup>a</sup> ; 001508/ KJ700889 <sup>a</sup> ; 001509/ KJ700890 <sup>a</sup> ; 001510/KJ700891 <sup>a</sup>	В
	000323/KJ700892 <sup>b</sup> ; 001137/KJ700893 <sup>a</sup> ; 001138/KJ700894 <sup>a</sup> ; 001512 <sup>c</sup> /KJ700895 <sup>a</sup> ; 001513 <sup>c</sup> /KJ700896 <sup>a</sup> ; 001447 <sup>c</sup> /KJ700897 <sup>a</sup>	Е
C. rhaphidophorae	001514 <sup>c</sup> /KJ700898 <sup>a</sup> ; 001516 <sup>c</sup> /KJ700899 <sup>a</sup> ; 001525 <sup>c</sup> /KJ700900 <sup>a</sup> ; 001530 <sup>c</sup> /KJ700901 <sup>a</sup>	С
	000349/KJ700902 <sup>b</sup> ; 000350/KJ700903 <sup>b</sup> ; 000353/KJ700904 <sup>b</sup>	F
C. longifilamentata sp. n.	001515 <sup>c</sup> /KJ700905 <sup>a</sup> ; 001523 <sup>c</sup> /KJ700906 <sup>a</sup> ; 001526–1529 <sup>c</sup> /KJ700907–0910 <sup>a</sup> ; 001531–1533 <sup>c</sup> /KJ700911–0913 <sup>a</sup> ; 001576 <sup>c</sup> /KJ700914 <sup>a</sup> ; 001577 <sup>c</sup> /KJ700915 <sup>a</sup>	С
C. longivalva sp. n.	000082/KJ700916 <sup>b</sup> ; 000086/KJ700917 <sup>b</sup>	F
C. hailini sp. n.	000355-0358/KJ700918-0921 <sup>b</sup> ; 001298-1301 <sup>c</sup> /KJ700922-0925 <sup>a</sup>	F
	001448°/KJ700926°; 001450°/KJ700927°; 001517/KJ700928°	С
C. yini sp. n.	000160/KJ700929 <sup>b</sup> ; 000364/KJ700930 <sup>a</sup> ; 001185 <sup>c</sup> /KJ700931 <sup>a</sup> ; 001194/ KJ700932 <sup>a</sup> ; 001302 <sup>c</sup> /KJ700933 <sup>a</sup> ; 001312 <sup>c</sup> /KJ700934 <sup>a</sup>	F
	001586°/KJ700935°	С

**Table 2.** Specimens of *Colocasiomyia* species used for DNA barcoding analysis.

<sup>a</sup> PCR/sequencing using the primers of Folmer et al. (1994); <sup>b</sup> PCR/sequencing using the primers of Hebert et al. (2004); <sup>c</sup> Adults obtained by laboratory rearing; <sup>d</sup> Collection sites: A, Enrekang, South Sulawesi, Indonesia; B, Park Headquarters, Mt. Kinabalu, Sabah, Malaysia; C, Yixiang, Pu'er, Yunnan, China; D, Bogor Botanical Garden, West Java, Indonesia; E, Menglun, Mengla, Xishuangbanna, Yunnan, China; F, Baihualing, Longyang, Baoshan, Yunnan, China

## **DNA** barcoding

A total of 54 individuals representing all the three known and four morphologically identified, putatively new species (Table 2) were subjected to DNA sequencing of the *COI* barcode fragments (Hebert et al. 2003). In addition, three more species, *C. colocasiae* (Duda, 1924), *C. sulawesiana* Okada & Yafuso, 1989 and *C. xenalocasiae* (Okada, 1980) from the sister *cristata* group were included, for comparison of sequences. DNA was extracted using small piece(s) of abdominal tissue or the right hindleg of a single adult using the TIANamp® Genomic DNA Kit. The primer pair used for the PCR and sequencing of the *COI* fragment was either that designed by Folmer et al. (1994, LCO1490: 5'- GGTCAA CAAAT CATAA AGATA TTGG -3', HCO2198: 5'- TAAAC TTCAG GGTGA CCAAA AAATC A -3') or that by Hebert et al. (2004, LepF1: 5'- ATTCA ACCAA TCATA AAGAT ATTGG -3', LepR1: 5'- TAAAC TTCTG GATGT CCAAA AAATC A -3'). The 20 μl PCR reaction volume contains 0.1 μl *TaKaRa Ex Taq*® (5 U/μl), 0.4 μl of each primer (10 μM), 2 μl 10× *Ex Taq* Buffer (Mg\* Plus), 1.6 μl dNTP mixture (2.5 mM for each), 15 μl ddH<sub>2</sub>O and 0.5

µl of template DNA. The amplification of the *COI* fragments was initiated by 3-min pre-denaturation at 95 °C, followed by 35 thermocycles of 40 s of denaturation at 94 °C, 50 s of annealing at 46 °C, and 1 min of extension at 72 °C, ended by a 5 min of post-extension at 72 °C. The PCR products were purified and then subjected to DNA sequencing on an ABI® 3730 DNA Analyser.

A total of 57 COI sequences (54 of the *gigantea* group and three of the *cristata* group) were determined. The sequences were edited in the SEQMAN module of the DNASTAR package (DNASTAR Inc. 1996), and aligned in MEGA5 (Tamura et al. 2011). Then the inter- and intraspecific genetic distances were calculated for the species of the *gigantea* group using the Kimura 2-parameter (K2P) model in MEGA5. In addition, we also conducted a character-based species barcoding. Since there were end gaps (missing data) in some of the sequences of each species, the end sites with an overlapping of less than three (two for *C. longivalva*) sequences were excluded. The sites being fixed within a species but differing from the other species (including the three species of *cristata* group) were manually selected as diagnostic sites (i.e., "pure" diagnostics, Sarkar et al. 2002, DeSalle et al. 2005), for each species.

#### Results

## **DNA** barcoding

The alignment of the 57 *COI* sequences spanned 658 nucleotide sites in length, with 184 variable sites, among which 160 were parsimony informative. For the inter- and intraspecific K2P distances see Table 3. The largest intraspecific K2P distance in the *gigantea* group was found in *C. scindapsae* (= 0.0102), while the smallest interspecific one was found between *C. rhaphidophorae* and *C. longifilamentata* (= 0.0135). This implies that the "barcoding gap" (Meyer and Paulay 2005) is too narrow, only 0.0033, to validate the distance-based species delimitation in the *gigantea* group.

Fig. 1 shows nucleotides at the sites where "pure" diagnostics for any species of the *gigantea* group are included. At least one diagnostic site was recognized for each species. For example, the site 226 is diagnostic for *C. rhaphidophorae*: this site has a fixed status of C (Cytosine) in this species, but T (Thymidine) in the other species. The sites 136 and 505 (both with fixed status of C) are diagnostic for *C. longifilamentata*.

# **Taxonomy**

Colocasiomyia gigantea species group Fartyal et al. (2013).

Included species. gigantea (Okada, 1987); rhaphidophorae Gao & Toda in Fartyal et al. 2013; scindapsae Fartyal & Toda in Fartyal et al. 2013; longifilamentata Li & Gao, sp. n.; longivalva Li & Gao, sp. n.; hailini Li & Gao, sp. n.; yini Li & Gao, sp. n.

Table 3. Intra- and interspecific K2P distances (minimum-maximum) in the C. gigantea species group.

	N.	Intraspecific			Interspe	Interspecific distance		
Species	<b>\</b>	distance	gigantea	scindapsae	rhaphidophorae	rhaphidophorae longifilamentata sp. n. longivalva sp. n. hailini sp. n.	longivalva sp. n.	bailini sp. n.
gigantea	4	8500.0-0						
scindapsae	9	0-0.0102	0.1027-0.1236					
rhaphidophorae	10	0-0.0054	0.1056-0.1148 0.0978-0.1156	0.0978-0.1156				
longifilamentata sp. n.	14	6600.0-0	0.1134-0.1259	$0.1134 - 0.1259 \; \left  \; 0.0972 - 0.1088 \; \right  \; 0.0135 - 0.0282$	0.0135-0.0282			
longivalva sp. n.	2	0-0	0.1345-0.1370	0.1345 - 0.1370  0.0930 - 0.1046  0.0793 - 0.0860	0.0793-0.0860	0.0815-0.0834		
hailini sp. n.	11	6900.0-0	0.1099-0.1179	0.1099 - 0.1179  0.1017 - 0.1230  0.0939 - 0.1038	0.0939-0.1038	0.0978-0.1086	0.1079-0.1125	
<i>yini</i> sp. n.	7	0-0.0034	0.1440-0.1483	$.0034  \left  \begin{array}{c c} 0.1440 - 0.1483 & 0.1425 - 0.1664 & 0.1211 - 0.1359 \end{array} \right.$	0.1211-0.1359	0.1367-0.1484	0.1431–0.1468   0.0674–0.0758	0.0674-0.0758

a Number of sequences

 $\begin{array}{c} 122222222222233333334444444555555555\\ 7830122345677801445780355689011234579\\ 6565169879847049675657614049517954372 \end{array}$ 

	0303109079047049073037014049317934372
C. sulawesiana	Lot055 ATTTATATTTTTTAAAATATTTACATTTTAATTTAAA
C. colocasiae	Lot 072 A T . TT
C. xenalocasiae	
	001627 . A . A
C. gigantea	Lot150 .AAAC.T.TC.TA.CGG.CGTAA.C
	001444 .GAAC.T.TC.TA.CGG.CGTAA.C
	001445 .AAAC.T.TC.TA.CGG.CGTAA.C
	001449 .AAAC.T.TC.TA.CGG.CGTAA.C
C. scindapsae	
C. Scilldapsae	
	Lot037 C T. CT. TC A A TA
	001186CT.CT.TCAATA
	001508CT.CT.TAAATA
	001509 C T . C T . T .
	001510 C T. CT. TC A A TA
C rhanhidanharaa	
C. rhaphidophorae	000323CA.T.TACTAA
	001137CA.T.TACTAA
	001138 C A . T . T AC TAA
	001512CA.T.TACTAA
	001513 C A . T . T AC TAA
	001447 C A.T.T AC TAA
	001514 C A . T . T AC TAA
	001516 C A . T . T A C TAA
	001525CA.T.TACTAA
	001530CA.T.TACTAA
C. longifilamentata	000349CA.T.TACC.TAA
3	000350CA.T.TACC.TAA
	000353CA.T.TACC.TAA
	001515 C
	001523 C
	001526CA.T.TACC.TAA
	001527 C A.T.T AC C.TAA
	001528 C A . T . T A C C . TAA
	001529 C A . T . T AC C . TAA
	001531CA.T.TACC.TAA
	001532CA.T.TACC.TAA
	001533 . C A.T.T AC C.TAA
0 1	001577CA.T.TACC.TAA
C. longivalva	000082 TCACA.C.TTACAGA.G.
	000086 TCACA.C.TTACAGA.G.
C. hailini	000355 .AGA.T.TC.TTCA
	000356 .AGA.T.TC.TTCA
	000357 .AGA.T.TC.TTCA
	000358 .AGA.T.TC.TTCA
	001299 .AGA.T.TC.TTCA
	001300 .AGA.T.TC.TTCA
	001301 .AGA.T.TC.TTCA
	001448 .AGA.T.TC.TTCA
	001450 .AGA.T.TC.TTCA
	001517 .AGA.T.TC.TTCA
C. yini	
O. yiiii	
	000364 .A.CGC.ACTGTGCC.CTG.CACG
	001185 .A.CGC.ACTGTGCC.CTG.CACG
	001194 .A.CGC.ACTGTGCC.CTG.CACG
	001302 .A.CGC.ACTGTGCC.CTG.CACG
	001312 .A.CGC.ACTGTGCC.CTG.CACG
	001586 .A.CGC.ACTGTGCC.CTG.CACG

**Figure 1.** Diagnostic nucleotide sites in the alignment of *COI* sequences of the *C. gigantea* group. Numbers at the top show the positions of the sites in the *COI* alignment (658-bp in length). Shaded sites are diagnostic for each species. Hyphens (-) indicate end missing data.

**Diagnosis** (Fartyal et al. 2013). Foreleg second tarsomere with 6–11 heavily sclerotized, nearly equally-sized pegs in 2 rows on ventro-apical elongation (Figs 2–15). Epandrium with very large, lobe-like apodeme on antero-subdorsal to -ventral margin (Figs 24, 30, 38, 45). Lateral lobes of oviscapt fused to each other only apically, with large patch of dense, distinct warts on basal half (Figs 16–22, 28, 36, 43, 50).

**Remarks.** The characters described to be common among the three known species of the *gigantea* group by Fartyal et al. (2013) are shared also by the four new species to be described here, except for the distance between antennal sockets larger than the socket width: the former is as large as the latter in *C. hailini* sp. n.

## Colocasiomyia longifilamentata Li & Gao, sp. n.

http://zoobank.org/67051239-8955-4D5D-A7CE-9B575A4270A9 http://species-id.net/wiki/Colocasiomyia\_longifilamentata Figs 5, 12, 19, 23–28

**Type material.** Holotype ♂ (No. 000068): CHINA: Baihualing, Longyang, Baoshan, Yunnan, 1500 m (25°17.19'N, 98°47.65'E), *ex Rhaphidophora decursiva* inflorescence at Stage III (the male phase: stamens appearing on the surface of spadix and dehiscing to release pollen), 16.vi.2011, JJ Gao (KIZ).

Paratypes: same data as holotype  $(73, 19; Nos\ 000069-78, 290, 291, 293)$ ; same but  $16-17.vi.2011\ (33, 19; Nos\ 000349, 350, 1133, 1134)$ ; same but  $12.vii.2011\ (13; No.\ 000168)$ ; same but  $14.vii.2011\ (13; No.\ 000353)$ ; same but from laboratory rearing of eggs in infructescences of *R. decursiva* collected on 16.vi.2011, JJ Gao  $(43, 19; Nos\ 001597, 98, 1631-33)$ ; from laboratory rearing of eggs in infructescences of *R. decursiva* collected from Yixiang, Simao, Pu'er, Yunnan,  $1250\ m\ (22^\circ41.19'N, 101^\circ7.77'E)$ , on 12-13.xii.2012, JJ Gao and Z Fu  $(53, 59; Nos\ 001523, 26, 27-29, 31-33, 76, 77)\ (KIZ, SEHU)$ .

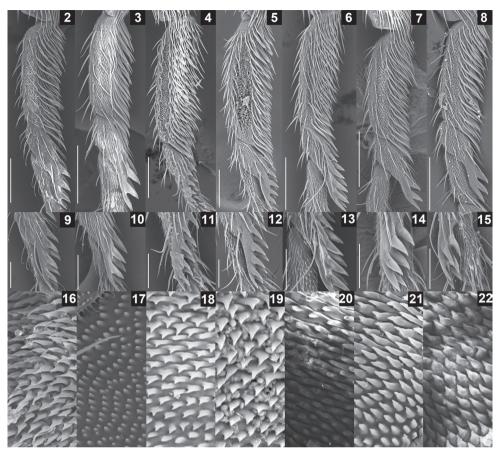
**Description.** Adult male. Head: Supracervical setae 12–15 per side. Dorsomedial arm of tentorial apodeme approximately 1/2 as long as dorsolateral arm. Eye red, somewhat roundish, with very sparse interfacetal setulae. Frontal vitta mat, black. First flagellomere not concave on inner margin. Facial carina trapeziform, medially wider than twice width of first flagellomere, as long as pedicel and first flagellomere combined. Palpus convex on ventrodistal portion. Cibarial posterior sensilla minute, 2 or 3 per side (Fig. 23). Labellum with 22 pseudotracheae per side.

Thorax: Scutum and scutellum glossy, blackish brown to black; thoracic pleura glossy, blackish brown. Acrostichal setulae in 6 rows.

Wing: Veins yellow. Halter grayish brown except for grayish yellow stalk.

Legs: Foreleg second tarsomere with 8–11 pegs (Figs 5, 12). Foreleg coxa large, with approximately 8 long setae on underside near attachment to trochanter. Small preapical dorsal setae present only on tibiae of hindlegs.

Abdomen: Tergites glossy, entirely blackish brown; II to VI+VII each bearing setulae and setae in approximately 3–4 transverse rows; setae of posteriormost row largest. Sternites pale brown to brown; VI posteriorly not bilobed.



Figures 2–22. SEM photographs showing leg and oviscapt fine structures in the *Colocasiomyia gigantea* species group. Foreleg tarsomeres I and II (2–8), pegs on foreleg tarsomere II (9–15) and warts on basal part of lateral lobe or basal membrane of oviscapt (16–22) of *C. gigantea* (2, 9, 16), *C. scindapsae* (3, 10, 17), *C. rhaphidophorae* (4, 11, 18), *C. longifilamentata* sp. n. (5, 12, 19), *C. longivalva* sp. n. (6, 13, 20), *C. hailini* sp. n. (7, 14, 21) and *C. yini* sp. n. (8, 15, 22). Scale line = 0.1 mm in 2–8, 0.05 mm in 9–15. Figures 16–22 are in the same magnification, with the width corresponding to 30 µm.

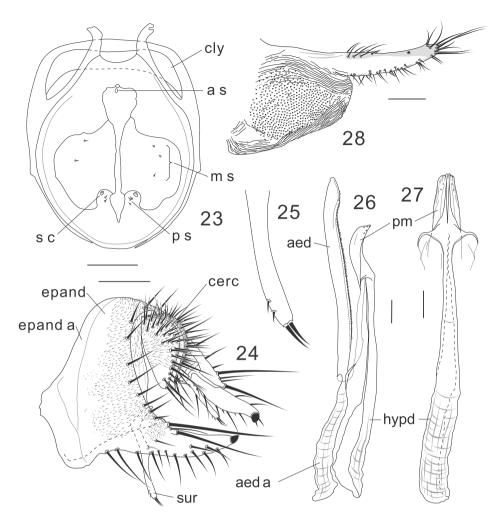
Terminalia (Figs 24–27): Epandrium pubescent except for anterior margin, anteroand postero-ventral portion and large apodeme lobe, with 6–7 setae per side near posterior margin; anteroventral portion of epandrium curved inward, apically articulated to lateral arm of hypandrium; posteroventral lobe well developed, narrowly prolonged, scabbard-like, apically with a large peg, latero-ventrally with 12–13 short to moderate setae, dorso-subbasally with 2 very long, apically diverged setae extending almost beyond its tip, and 2 shorter ones (Fig. 24). Cercus crescent, pubescent on dorsal 2/3, with approximately 31 long setae, including 3 distinctly longer ones: 2 on ventral apex and 1 on subventral portion (Fig. 24). Membrane between epandrium and cercus pubescent dorsally to laterally. Surstylus entirely narrow, downward elongated, with only trichoid setae: 2 long at apex, 1 long on submedial inner surface, and 2 small setulae on subapical outer surface (Figs 24, 25). Median piece of 10th sternite somewhat anchor-shaped in posterior view, medially forming longitudinal ridge, laterally with broad flank. Paramere somewhat blade-like in lateral view, apically with 5 minute sensilla along edge (Figs 26, 27). Aedeagus nearly entirely separated into a pair of lateral lobes ventrally connected by subbasally to subapically densely pubescent membrane, slightly curved ventrad subapically, somewhat pointed apically; apodeme proceeding nearly along aedeagal axis, shorter than aedeagus, but longer than its 1/2; aedeagal basal processes connecting dorsobasal corners of aedeagus and lateral arms of hypandrium (Fig. 26).

Measurements (holotype / range in 6% paratypes, in mm): BL (straight distance from anterior edge of pedicel to tip of abdomen) = 2.76 / 2.45–2.75, ThL (medial distance from anterior notal margin to apex of scutellum) = 1.29 / 1.17–1.37, WL (distance from humeral cross vein to wing apex) = 2.49 / 2.31–2.74, WW (maximum wing width) = 1.08 / 0.96–1.07.

Indices (holotype / range in 6♂ paratypes): FW/HW (frontal width / head width) = 0.55 / 0.55 - 0.57, ch/o (maximum width of gena / maximum diameter of eye) = 0.52/0.44-0.63, prorb (proclinate orbital seta / posterior reclinate orbital seta in length) = 1.55 / 1.34–1.67, rcorb (anterior reclinate orbital seta / posterior reclinate orbital seta in length) = 0.49 / 0.38-0.56, orbito (distance between proclinate and posterior reclinate orbital setae / distance between inner vertical and posterior reclinate orbital setae) = 0.71 / 0.70-0.83, vb (subvibrissal seta / vibrissa in length) = 0.33 / 0.24-0.39, dcl (anterior dorsocentral seta / posterior dorsocentral seta in length) = 0.51 / 0.53-0.68, presctl (prescutellar seta / posterior dorsocentral seta in length) = 0.46 / 0.45–0.54, sctl (basal scutellar seta / apical scutellar seta in length) = 0.71 / 0.63-0.74, sterno (anterior katepisternal seta / posterior katepisternal seta in length) = 0.72 / 0.68-0.80, mid katepisternal seta indistinguishable from the other fine setae, dcp (distance between ipsilateral dorsocentral setae / distance between anterior dorsocentral setae) = 1.07 / 0.85-1.31, sctlp (distance between ipsilateral scutellar setae / distance between apical scutellar setae) = 1.10 / 0.94-1.39, C (2nd costal section between subcostal break and  $R_{2+3}$  / 3rd costal section between  $R_{2+3}$  and  $R_{4+5}$ ) = 2.56 / 2.28-2.75, 4c (3rd costal section between  $R_{2+3}$  and  $R_{4+5}$  /  $M_1$  between r-m and dm-cu) = 0.81 / 0.76-0.98, 4v ( $M_1$ between dm-cu and wing margin / M<sub>1</sub> between r-m and dm-cu) = 1.44 / 1.37-1.70,  $5 \times (CuA_1 \text{ between dm-cu and wing margin / dm-cu between } M_1 \text{ and } CuA_1) = 0.88 /$ 0.75–0.82, ac (3rd costal section between  $R_{2,3}$  and  $R_{4,5}$  / distance between distal ends of  $R_{4.5}$  and  $M_1$ ) = 3.44 /2.81–4.13, M (CuA<sub>1</sub> between dm-cu and wing margin /  $M_1$ between r-m and dm-cu) = 0.16 / 0.14-0.19, C3F (length of heavy setation in 3rd costal section / length of 3rd costal section) = 0.79 / 0.74 - 0.83.

**Female.** Head, thorax, wing and legs as in male.

Terminalia: Tergite VII mid-dorsally not constricted; VIII entirely pubescent, with 5 setae in transverse (against body axis) row on discolored posteroventral portion. Oviscapt distally narrowing; distal narrow portion as long as proximal, broad portion, with approximately 15, 6 and 3 trichoid ovisensilla per side on ventral, dorsal and apical margins, respectively, and a tiny, peg-like ovisensillum near dorsosubapical margin (Fig. 28).



**Figures 23–28.** *Colocasiomyia longifilamentata* Li & Gao, sp. n. Adult male and female (paratypes) from Baihualing, Yunnan, China: **23** Cibarium and clypeus (dorsal view) **24** periphallic organs (posterolateral view) **25** apical part of surstylus **26** phallic organs (lateral view) **27** hypandrium and parameres (ventral view) **28** oviscapt (lateral view). Abbreviations: aed = aedeagus, aed a = aedeagal apodeme, a s = anterior sensilla, cerc = cercus, cly = clypeus, epand = epandrium, epand a = epandrial apodeme, hypd = hypandrium, m s = medial sensilla, ps = posterior sensilla, pm = paramere, s c = sensilla campaniformia, sur = surstylus. Scale lines = 0.1 mm.

Measurements (range in 5 paratypes, in mm): BL = 2.66–3.30, ThL = 1.33–1.47, WL = 2.40–2.90, WW =1.03–1.21.

Indices (range in 5 paratypes): FW/HW = 0.55–0.60, ch/o = 0.51–0.59, prorb = 1.35–1.56, rcorb = 0.42–0.78, orbito = 0.65–0.80, vb = 0.28–0.39 (4 $\$ ), dcl = 0.46–0.63, presctl = 0.45–0.56, sctl = 0.65–0.74 (4 $\$ ), sterno = 0.44–0.77, dcp = 0.92–1.00, sctlp = 1.04–1.20, C = 2.21–2.68, 4c = 0.79–0.90, 4v = 1.44–1.53, 5x = 0.68–0.97, ac = 2.73–3.59, M = 0.17–0.19, C3F = 0.78–0.86.

**Egg.** Filaments 2, approximately 1.8–2.4 times as long as length of egg body. **Etymology.** The specific name "*longifilamentata*" refers to the long filaments of egg. **Distribution.** China (Yunnan).

**Remarks.** Although this species closely resembles *C. rhaphidophorae* in the external morphology and structures of male and female terminalia, it can be easily distinguished from the latter by the epandrium having several setae on the dorsal to lateral portion (Fig. 24) (*C. rhaphidophorae* lacking setae there).

Colocasiomyia longivalva Li & Gao, sp. n.

http://zoobank.org/01776174-A214-4450-8AD6-8B17016CAA15 http://species-id.net/wiki/Colocasiomyia\_longivalva Figs 6, 13, 20, 29–36

**Type material.** Holotype ♂ (No. 000079): CHINA: Baihualing, Longyang, Baoshan, Yunnan, 1500 m (25°17.19'N, 98°47.65'E), *ex R. decursiva* inflorescence at Stage III, 17.vi.2011, JJ Gao (KIZ).

Paratypes: same data as holotype but 16.vi.2011 (2 $\circlearrowleft$ , 8 $\updownarrow$ : Nos 000080–87, 171, 172); same but 15.vi.2011 (1 $\circlearrowleft$ : No. 000170) (KIZ, SEHU).

**Description.** Adult male. Head: Supracervical setae 13 per side. Dorsomedial arm of tentorial apodeme 1/3 as long as dorsolateral arm. Eye red, somewhat roundish, with very sparse interfacetal setulae. Frontal vitta mat, black. First flagellomere not concave on inner margin. Facial carina trapeziform, medially wider than twice width of first flagellomere, as long as pedicel and first flagellomere combined. Palpus convex on ventrodistal portion. Cibarial posterior sensilla minute, 1 per side (Fig. 29). Labellum with 20 pseudotracheae per side.

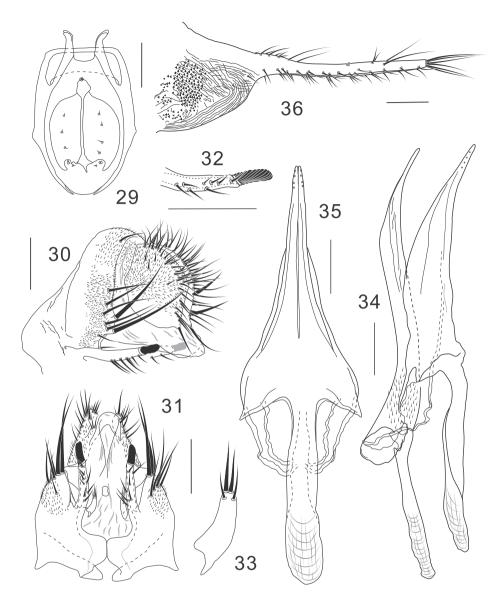
Thorax: Scutum and scutellum glossy, black; thoracic pleura glossy, blackish brown. Acrostichal setulae in 6 rows.

Wing: Veins yellow. Halter grayish brown except for grayish yellow stalk.

Legs: Foreleg second tarsomere with 8–10 pegs (Figs 6, 13). Foreleg coxa large, with approximately 8 long setae on underside near attachment to trochanter. Small preapical dorsal setae present only on tibiae of hindlegs.

Abdomen: Tergites glossy, entirely dark brown; II to VI+VII each bearing setulae and setae irregularly arranged; setae of posteriormost row largest. Sternites grayish yellow; VI posteriorly not bilobed.

Terminalia (Figs 30–35): Epandrium notched above insertion of ventral lobe, pubescent except for anterolateral margin to ventral portion and ventral lobe, with 7–8 setae per side along ventral margin of ventral lobe and approximately 10 setae (including 2 thickest ones located just above and 2 shortest ones just on subventral notch) along posterior margin; anteroventral portion curved inward, apically articulated to lateral arm of hypandrium (Figs 30, 31); ventral lobe prolonged like rod, apically with grooved, finger-like peg (Figs 30–32). Surstylus basally articulated to inner, basal corner of epandrial ventral lobe, 1/3 as long as epandrial ventral lobe, distally nearly parallel with epandrial ventral lobe,



**Figures 29–36.** Colocasiomyia longivalva Li & Gao, sp. n. Adult male and female (paratypes) from Baihualing, Yunnan, China: **29** Cibarium and clypeus (dorsal view) **30** periphallic organs (posterolateral view) **31** periphallic organs (ventral view) **32** apical part of epandrial ventral lobe (ventral view) **33** surstylus (ventral view) **34** phallic organs (lateral view) **35** hypandrium and parameres (ventral view) **36** oviscapt (lateral view). Scale lines = 0.1 mm.

with 2 trichoid setae apically and 2 trichoid, thinner setae on ventral, subapical surface (Figs 31, 33). Cercus large, somewhat rhombic, wider than 1/2 its (dorsoventral) height, pubescent on dorsal 2/3, with approximately 33 setae mostly distributed near posterior

margin, including slightly prominent one at caudoventral apex (Fig. 30). Membrane between epandrium and cercus pubescent dorsally to laterally. Tenth sternite folded into two lateral lobes connected with each other caudodorsally; lateral lobe triangularly extended anterodorsally, fused with membrane between epandrium and cercus. Hypandrium narrow plate-like, posteriorly T-shaped, with lateral arms fused to membranous, aedeagal basal processes (Figs 34, 35). Parameres long, coalescent to hypandrium, triangular in ventral view, apically with 4 minute sensilla arranged in a row, basally fused to each other (Figs 34, 35). Aedeagus nearly entirely separated into a pair of lateral lobes, pubescent basally, bent ventrad subapically, narrowly pointed at apex; aedeagal apodeme rod-like, 1/2 as long as aedeagus (Fig. 34); aedeagal basal processes membranous, connecting dorsobasal corners of aedeagus and lateral arms of hypandrium.

Measurements (holotype / range in  $3 \circlearrowleft$  paratypes, in mm): BL = 3.30 / 2.40 - 3.20, ThL = 1.40 / 1.18 - 1.42, WL = 2.80 / 2.50 - 2.88, WW = 1.09 / 1.03 - 1.20.

Indices (holotype / range in  $3\delta$  paratypes): FW/HW = 0.49 / 0.48-0.50, ch/o = 0.51 / 0.53-0.54, prorb = 1.04 / 1.25-1.49, rcorb = 0.44 / 0.44-0.50, orbito = 0.74 / 0.56-0.66, vb = 0.47 / 0.38-0.50, dcl = 0.50 / 0.51-0.59, presctl = 0.50 / 0.51-0.59, sctl = 0.91 / 0.65-0.77, sterno = 0.65 / 0.56-0.66, mid katepisternal seta indistinguishable from other fine setae, dcp = 1.08 / 0.86-1.07, sctlp = 1.23 / 1.10-1.26, C = 1.76 / 1.79-1.83, 4c = 1.16 / 1.18-1.19, 4v = 1.83 / 1.85-1.95, 5x = 0.98 / 0.99-1.18, ac = 4.54 / 3.84-4.08, M = 0.14 / 0.15-0.17, C3F = 0.83 / 0.84-0.88.

Female. Head, thorax, wing and legs as in male.

Terminalia: Tergite VII mid-dorsally not constricted; VIII pubescent nearly entirely, with 3–4 setae in a transverse row on unpubescent medio-posterior portion. Oviscapt distal narrow portion twice as long as proximal, broad portion, with approximately 18, 6 and 3 trichoid ovisensilla per side on ventral, dorsal and apical margins, respectively, and tiny, peg-like ovisensillum near mid-dorsal margin (Fig. 36).

Measurements (range in  $8^{\circ}$  paratypes, in mm): BL = 2.80-3.21 (7°), ThL = 1.26-1.47, WL = 2.67-2.90, WW =1.08-1.26.

Indices (range in 8 paratypes): FW/HW = 0.49-0.52, ch/o = 0.49-0.54, prorb = 0.97-1.25, rcorb = 0.34-0.57, orbito = 0.56-0.73, vb = 0.43-0.64, dcl = 0.49-0.56, presctl = 0.49-0.56, sctl = 0.64-0.87 (7 ), sterno = 0.63-0.87, dcp = 0.82-1.06, sctlp = 1.10-1.31, C = 1.78-2.06, 4c = 1.05-1.23, 4v = 1.81-1.99, 5x = 0.88-1.18, ac = 3.55-4.36, M = 0.14-0.18, C3F = 0.84-0.89.

**Distribution.** China (Yunnan).

Etymology. Pertaining to the long oviscapt valva.

**Remarks.** Adults of this species were very rarely captured from inflorescences of *R. decursiva*. So far we have never get any adult of this species by laboratory rearing from the host inflorescences/infructescences. This species is distinguished from the other members of the *gigantea* group: epandrium somewhat notched above insertion of ventral lobe; epandrial ventral lobe prolonged like rod, apically with grooved, finger-like peg (Figs 30–32); surstylus 1/3 as long as epandrial ventral lobe, distally nearly parallel with the latter (Figs 31, 33); paramere large, as long as hypandrium (Figs 34, 35); distal narrow portion of oviscapt twice as long as proximal, broad portion (Fig. 36).

Colocasiomyia hailini Li & Gao, sp. n.

http://zoobank.org/8F467F73-9310-499B-875B-AB023BCD6992 http://species-id.net/wiki/Colocasiomyia\_hailini Figs 7, 14, 21, 37–43

**Type material.** Holotype ♂ (No. 001641): CHINA: Baihualing, Longyang, Baoshan, Yunnan, 1500 m (25°17.27′N, 98°48.7′E), *ex R. decursiva* inflorescence at Stage III, 29.vi.2006, JT Yin (KIZ).

Paratypes: same data as holotype (11\$\int\_0\$, 27\$\infty\$: Nos 001634\$\\_-40\$, 42\$\\_-49\$, 51\$\\_-73); same but (25°17.19'N, 98°47.65'E) (2\$\int\_0\$, 2\$\infty\$: Nos 001674, 75, 1135, 1136), 19.vi.2011, JJ Gao; same but (25°17.19'N, 98°47.65'E) (4\$\int\_0\$: Nos 000355\$\\_-58), 12\$\\_-15.vii.2011, JJ Gao; same but (25°17.19'N, 98°47.65'E) (2\$\int\_0\$, 2\$\infty\$: Nos 001298\$\\_-301), from laboratory rearing of eggs in infructescences of *R. decursiva* collected on 3\$\\_-9\$. viii.2012, JJ Gao, Z Fu, NN Li, JM Chen and SS Li; Yixiang, Simao, Pu'er, Yunnan (22°41.19'N, 101°7.77'E) (2\$\int\_0\$, 1\$\infty\$: Nos 001448, 50, 1517), from laboratory rearing of eggs in infructescences of *R. decursiva* collected on 12\$\\_-13.xii.2012\$, JJ Gao and Z Fu (KIZ, SEHU).

**Description.** Adult male. Head: Supracervical setae approximately 7 per side. Dorsomedial arm of tentorial apodeme 1/3 as long as dorsolateral arm. Eye red, somewhat roundish, with very sparse interfacetal setulae. Frontal vitta mat black. First flagellomere concave on inner margin. Facial carina trapeziform, medially wider than twice width of first flagellomere, as long as pedicel and first flagellomere combined. Palpus convex on ventrodistal portion. Cibarial posterior sensilla minute, 1 per side (Fig. 37). Labellum with 11 pseudotracheae per side.

Thorax: Scutum and scutellum glossy, blackish brown to black; thoracic pleura glossy, blackish brown. Acrostichal setulae in 4 rows.

Wing: Veins yellow. Halter grayish brown except for grayish yellow stalk.

Legs: Foreleg second tarsomere with 6 pegs (Figs 7, 14). Foreleg coxa large, with approximately 15 long setae on underside near attachment to trochanter. Small preapical dorsal setae present only on tibiae of hindlegs.

Abdomen: Tergites glossy, entirely dark brown; II to VI+VII each bearing setulae and setae in approximately 3 transverse rows; setae of posteriormost row largest. Sternites pale brown to brown; VI posteriorly not bilobed.

Terminalia (Figs 38–42): Epandrium pubescent dorsomedially only, with 6 setae per side near posterior margin, 15–16 setae per side on ventral portion and 23–24 setae as thick as upper cercal ones along ventral margin of ventral lobe; apodeme well developed into distally tapering, triangular extension strongly projected anteriad, twice as long as epandrial width, broadly sclerotized along dorsal and ventral margins (Fig. 38); anteroventral portion of epandrium curved inward, apically articulated to lateral arm of hypandrium. Surstylus broad, basally narrowly fused to basal corner of epandrial ventral lobe, dorsally broadly sclerotized, with 1 short, trichoid seta on upper medial portion of outer surface and 3 large, peg-like prensisetae on distal margin; lowest prensiseta nearly as long as width of surstylus, curved inwards and slightly downwards, especially in distal

1/3, narrowly edged by caudoventral portion of surstylus only along its basal portion (Figs 38, 39). Cercus semilunar, narrower than 1/2 its (dorsoventral) height, pubescent on dorsal 2/3, with approximately 48 setae (including prominent one twice as long as others) all over and approximately 45 setulae on caudoventral, inner margin; ventral slightly incurved lobe 1/3–1/4 of cercal height (Fig. 38). Membrane between epandrium and cercus pubescent dorsally to laterally. Median piece of 10th sternite cordiform in posterior view, moderately sclerotized; lateral piece somewhat cuneiform, narrowing anteriad, connected to inner, basal corner of epandrial ventral lobe with membranous tissue. Hypandrium long, narrow, plate-like, anteriorly widened (Fig. 42). Parameres somewhat semilunar in ventral view, basally fused to each other, apically with 5 or 6 minute sensilla in small medioapical patch (Figs 40, 42). Aedeagus nearly entirely separated into a pair of lateral lobes, nearly straight, apically trilobed; lobes curved ventrad and connected with each other by tendon-like membranous structures (Figs 40, 41); apodeme rod-like, arched in lateral view, longer than aedeagus (Fig. 40).

Measurements (holotype / range in 10% paratypes, in mm): BL = 2.01 / 1.85 - 2.14, ThL = 0.86 / 0.76 - 0.94, WL = 1.79 / 1.59 - 1.89, WW = 0.79 / 0.72 - 0.85.

Indices (holotype / range in  $10 \ \$  paratypes): FW/HW =  $0.54 \ / 0.53-0.56$ , ch/o =  $0.48 \ / 0.46-0.55$ , prorb = broken /  $1.08 \ (1 \ \ )$ , rcorb =  $0.61 \ / 0.56-0.59 \ (6 \ \ )$ , orbito =  $0.86 \ / 0.63-0.85$ , vb =  $0.31 \ / 0.30-0.36$ , dcl =  $0.52 \ / 0.54-0.62 \ (6 \ \ )$ , presctl =  $0.38 \ / 0.30-0.39 \ (9 \ \ )$ , sctl =  $0.75 \ / 0.59-0.77 \ (9 \ \ )$ , sterno =  $0.75 \ / 0.72-0.94$ , mid katepisternal seta indistinguishable from other fine setae, dcp =  $0.92 \ / 0.82-1.02$ , sctlp =  $1.00 \ / 0.92-1.12$ , C =  $1.90 \ / 1.72-2.12$ , 4c =  $1.18 \ / 0.89-1.21$ , 4v =  $1.79 \ / 1.28-1.75$ , 5x =  $1.15 \ / 0.94-1.17$ , ac =  $3.56 \ / 3.04-3.59$ , M =  $0.19 \ / 0.19-0.21$ , C3F =  $0.48 \ / 0.35-0.53$ .

Female. Head, thorax, wing and legs as in male.

Terminalia: Tergite VII mid-dorsally not constricted; VIII pubescent nearly entirely, with 4–5 setae in a longitudinal row on unpubescent, discolored, posteroventral portion. Oviscapt distal elongation constricted dorsally at basal 1/3, apically somewhat truncate, with 6, 9 and 3 trichoid ovisensilla per side on distal 1/3 of dorsal margin, entire ventral margin, and at apex, respectively (Fig. 43).

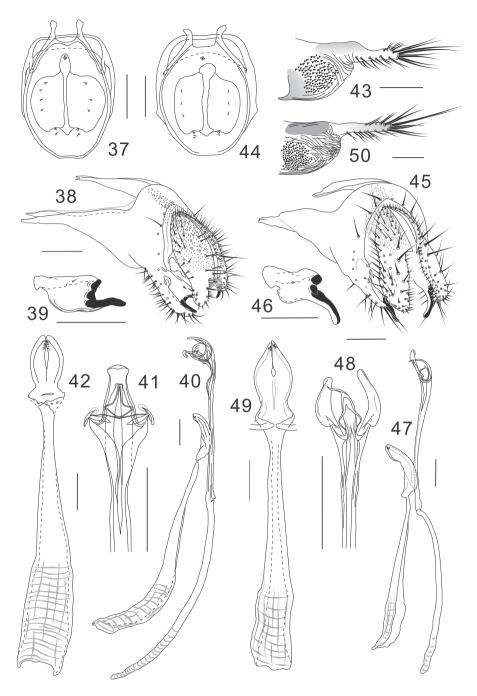
Measurements (range in 10 paratypes, in mm): BL = 2.43–2.95, ThL = 0.89–1.22, WL = 1.90–2.48, WW = 0.85–1.07.

Indices (range in 10 paratypes): FW/HW = 0.55–0.59, ch/o = 0.50–0.62, prorb broken, rcorb = 0.40–0.52 (3 $\updownarrow$ ), orbito = 0.68–0.91, vb = 0.29–0.35, dcl = 0.43–0.57 (6 $\updownarrow$ ), presctl = 0.29–0.37 (7 $\updownarrow$ ), sctl = 0.69–0.77 (9 $\updownarrow$ ), sterno = 0.74–0.91, dcp = 0.90–1.09, sctlp = 1.00–1.12, C = 1.83–2.23, 4c = 1.00–1.14, 4v = 1.50–1.86, 5x = 0.87–1.19, ac = 3.28–4.00, M = 0.16–0.20, C3F = 0.38–0.59.

**Distribution.** China (Yunnan).

**Etymology.** The specific name "hailini" is a patronym in honor of the emeritus Professor Hai-lin Wang of the Southwest Forestry University, China.

**Remarks.** This species resembles the following species, *C. yini* sp. n., in overall outer morphology, but can be distinguished from the latter by 1) C3F < 2/3 (C3F  $\ge 2/3$  in *yini* sp. n.); 2) lowest, peg-like prensiseta on distal margin of surstylus curved inwards and



**Figures 37–50.** Colocasiomyia hailini Li & Gao, sp. n. (**37–43**) and *C. yini* Li & Gao, sp. n. (**44–50**). Adult males and females (paratypes) from Baihualing, Yunnan, China: **37, 44** Cibarium and clypeus (dorsal view) **38, 45** periphallic organs (posterolateral view) **39, 46** surstylus **40, 47** phallic organs (lateral view) **41, 48** apical part of aedeagus (ventral view) **42, 49** hypandrium and parameres (ventral view) **43, 50** oviscapt (lateral view). Scale lines = 0.1 mm.

slightly downwards, especially in distal 1/3, narrowly edged by caudoventral portion of surstylus only along its basal portion (Fig. 39) [in *C. yini* sp. n., lowest peg-like prensiseta strongly curved downwards in distal half, narrowly edged by caudoventral portion of surstylus along its whole length (Figs 46)]; 3) ventral slightly incurved lobe of cercus 1/3–1/4 of cercal height (Fig. 38) [in *C. yini*, ventral lobe of cercus approximately 1/2 of cercal height (Fig. 45)]; 4) distal, narrow part of oviscapt constricted dorsally at basal 1/3 (Fig. 43) [in *C. yini* sp. n., distal, narrow part of oviscapt almost even-edged (Fig. 50)].

## Colocasiomyia yini Li & Gao, sp. n.

http://zoobank.org/0A22EA8C-6EF6-48DF-B46C-B946CEE327E9 http://species-id.net/wiki/Colocasiomyia\_yini Figs 8, 15, 22, 44–50

**Type material.** Holotype ♂ (No. 000159): CHINA: Baihualing, Longyang, Baoshan, Yunnan, 1500 m (25°17.19′N, 98°47.65′E), *ex R. decursiva* inflorescence at Stage III, 12.vii.2011, JJ Gao (KIZ).

Paratypes. Same data as holotype  $(33, 29: Nos\ 000160, 165-167, 178)$ ; same but  $16.vi.2011\ (13, 29: Nos\ 000161-163)$ ; same but  $(25^{\circ}17.27'N, 98^{\circ}48.7'E)$ ,  $29.vi.2006\ (19: No.\ 001650)$ , JT Yin; same but  $13.vii.2011\ (19: No.\ 000164)$ ; same but  $14.vii.2011\ (13: No.\ 000169)$ ; same but from laboratory rearing of eggs in infructescences of *R. decursiva* collected on 23-24.ix.2012, JJ Gao, Z Fu and JM Chen  $(19: No.\ 001185)$ ; from laboratory rearing of eggs in infructescences of *R. decursiva* collected from Yixiang, Simao, Pu'er, Yunnan  $(22^{\circ}41.19'N,\ 101^{\circ}7.77'E)$  on  $12-13.\ xii.2012$ , JJ Gao and Z Fu  $(19: No.\ 001586)$  (KIZ, SEHU).

**Description.** Adult male. Head: Supracervical setae 11 per side. Dorsomedial arm of tentorial apodeme 1/3 as long as dorsolateral arm. Eye red, somewhat roundish, with very sparse interfacetal setulae. Frontal vitta mat black. First flagellomere not concave on inner margin. Facial carina trapeziform, medially wider than twice width of first flagellomere, as long as pedicel and first flagellomere combined. Palpus convex on ventrodistal portion. Cibarial posterior sensilla minute, 1 or 2 per side (Fig. 44). Labellum with 11 pseudotracheae per side.

Thorax: Scutum and scutellum glossy, black; thoracic pleura glossy, blackish brown. Acrostichal setulae in 4 rows.

Wing: Veins yellow. Halter dark brown except for grayish yellow stalk.

Legs: Foreleg second tarsomere with 6 pegs (Figs 8, 15). Foreleg coxa large, with approximately 10 long setae on underside near attachment to trochanter. Small preapical dorsal setae present only on tibiae of hindlegs.

Abdomen: Tergites glossy, entirely dark brown; II to VI+VII each bearing setulae and setae in approximately 3 transverse rows; setae of posteriormost row largest. Sternites II–V pale brown; VI blackish brown, and bilobed posteriorly.

Terminalia (Figs 45–49): Epandrium with 6 setae per side from lateral portion to middorsal, posterior margin, 14 setae per side in ventral portion and 15–16 setae as

thick as upper cercal ones along ventral margin of ventral lobe; apodeme well developed into distally tapering, triangular extension strongly projected anteriad, twice as long as epandrial width, broadly sclerotized along dorsal and ventral margins (Fig. 45); anteroventral portion of epandrium curved inward, apically articulated to lateral arm of hypandrium (Fig. 45). Surstylus basally fused to basal corner of epandrial ventral lobe, dorsally broadly sclerotized, with 1 short, trichoid seta on upper medial portion, and 3 large, peg-like prensisetae on distal margin; lowest prensiseta slightly longer than width of surstylus, strongly curved downwards in distal half, narrowly edged by caudoventral portion of surstylus along its whole portion (Figs 45, 46). Cercus oblong, narrower than 1/2 its (dorsoventral) height, pubescent on dorsal 1/2, with approximately 58 setae (including one distinctively longer than others) all over and approximately 26 setulae on caudoventral, inner margin; ventral lobe approximately 1/2 of cercal height (Fig. 45). Membrane between epandrium and cercus pubescent dorsally to laterally. Median piece of 10th sternite rhombic in posterior view, moderately sclerotized; lateral piece somewhat cuneiform, narrowing anteriad, connected to inner, basal corner of epandrial ventral lobe with membranous tissue. Hypandrium long, narrow, plate-like, anteriorly widened, posteriorly T-shaped, with lateral arms fused to membranous aedeagal basal processes (Figs 47, 49). Parameres somewhat semilunar in ventral view, basally fused to each other, apically with 6 minute sensilla in small patch (Figs 47, 49). Aedeagus nearly entirely separated into a pair of lateral lobes, slightly bent, apically trilobed; median lobe curved ventrad and connected with lateral ones by tendon-like membranous structures (Figs 47, 48); apodeme rod-like, arched in lateral view, as long as aedeagus (Fig. 47); aedeagal basal processes membranous, connecting dorsobasal corners of aedeagus and posterolateral expansions of hypandrium.

Measurements (holotype / range in 5 $\circlearrowleft$  paratypes, in mm): BL = 2.66 / 2.20–2.56 (4 $\circlearrowleft$ ), ThL = 1.12 / 0.90–1.05, WL = 2.27 / 1.87–2.27, WW =1.03 / 0.85–1.00.

Indices (holotype / range in  $5 \circlearrowleft$  paratypes): FW/HW = 0.53 / 0.50-0.56, ch/o = 0.54 / 0.47-0.59, prorb = 1.04 / 0.97-1.20, rcorb = 0.42 / 0.48-0.55, orbito = 0.58 / 0.56-0.68, vb = 0.46 / 0.40-0.52, dcl = 0.50 / 0.47-0.50 , presctl = 0.34 / 0.24-0.37, sctl = 0.62 / 0.57-0.69 ( $4 \circlearrowleft$ ), sterno = 0.79 / 0.79-1.21 ( $3 \circlearrowleft$ ), mid katepisternal seta indistinguishable from other fine setae, dcp = 0.88 / 0.80-0.90, sctlp = 1.15 / 0.90-1.03, C = 2.35 / 2.04-2.52, 4c = 0.96 / 0.95-1.07, 4v = 1.67 / 1.63-1.92, 5x = 1.02 / 1.06-1.27, ac = 3.75 / 3.00-3.68, M = 0.15 / 0.16-0.18, C3F = 0.77 / 0.69-0.80.

Female. Head, thorax, wing and legs as in male.

Terminalia: Tergite VII mid-dorsally not constricted; VIII pubescent nearly entirely, with 5–6 setae in a transverse row on unpubescent, discolored, posteroventral portion. Oviscapt distal elongation almost smooth on dorsal margin, with 5, 7 and 3 trichoid ovisensilla per side on distal 1/2 of dorsal margin, entire ventral margin and at apex, respectively, of distal elongation (Fig. 50).

Measurements (range in  $5^{\circ}$  paratypes, in mm): BL = 2.05–2.86 (4°), ThL = 0.93–1.20, WL = 2.20–2.60, WW = 0.86–1.05.

Indices (range in  $5^{\circ}$  paratypes): FW/HW = 0.53-0.56, ch/o = 0.43-0.60, prorb = 0.88-1.11, rcorb = 0.28-0.51, orbito = 0.60-0.70, vb = 0.29-0.45, dcl = 0.46-0.52,

presctl = 0.26-0.34, sctl = 0.50-0.65 (4  $\circlearrowleft$ ), sterno = 0.76-0.83 (4  $\circlearrowleft$ ), dcp = 0.81-0.84, sctlp = 0.85-1.13, C = 2.20-2.34, 4c = 0.96-1.03, 4v = 1.67-1.92, 5x = 0.91-1.23, ac = 3.12-4.76, M = 0.13-0.18, C3F = 0.65-0.74.

**Distribution.** China (Yunnan).

**Etymology.** In honor of Mr Jian-Tao Yin of the Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences.

**Remarks.** Much fewer adults of this species were collected from inflorescences of *R. decursiva* in comparison to *C. hailini* sp. n. and *C. longifilamentata* sp. n. Breeding of this species on *R. decursiva* was confirmed by laboratory rearing of eggs laid on inflorescences of the host plant. See the Remarks for *C. hailini* sp. n. with respect to morphological differences from it.

## A key to species of the Colocasiomyia gigantea species group

In this key, the numbers of figures of Fartyal et al. (2013) are given in double quotation marks.

1	Labellum with 16 pseudotracheae per side. Aedeagal apodeme distinctly
	longer than aedeagus ("Fig. 4C"). Distal, narrow part of oviscapt narrowing
	and gently curved ventrad, apically arrowhead-shaped ("Fig. 4E")
_	Labellum with ≤ 14 or ≥ 20 pseudotracheae per side. Aedeagal apodeme as
	long as or shorter than aedeagus (Figs 26, 34, 40, 47; "Figs 2F, 3D"). Distal
	narrow part of oviscapt truncate apically, or curved dorsad if not truncate2
2	Labellum with 14 pseudotracheae per side. Distal, narrow part of oviscapt
	broadly truncate apically, much shorter than proximal, broad part ("Fig. 2H")
_	Labellum with 11 or ≥ 20 pseudotracheae per side. Distal, narrow part of
	oviscapt not or only slightly truncate apically, curved dorsad apically, longer
	or only slightly shorter than proximal, broad part (Figs 28, 36, 43, 50)3
3	Labellum with 11 pseudotracheae per side. Foreleg tarsomere II with 6 pegs
	(Figs 7, 8, 14, 15). Acrostichal setulae in 4 rows4
_	Labellum with ≥ 20 pseudotracheae per side. Foreleg tarsomere II with ≥ 8
	pegs (Figs 2–6, 9–13). Acrostichal setulae in 6 rows5
4	Wing C3F index < 2/3. Distance between antennal sockets same as socket
	width. Distal, narrow part of oviscapt constricted subbasally on dorsal margin
	(Fig. 43)
_	Wing C3F index > 2/3. Distance between antennal sockets larger than socket
	width. Distal, narrow part of oviscapt finger-like, not constricted subbasally
	on dorsal margin (Fig. 50)
5	Epandrium notched above basal corner of epandrial ventral lobe; ventral lobe
-	prolonged like rod, apically with grooved, finger-like peg (Figs 30–32). Sur-
	protonged the roa, apically with grooved, miger like peg (1188 30 32). Our

### **Discussion**

DNA barcoding has been innovated to facilitate works of not only taxonomists but also non-experts in specimen identification (http://ibol.org/about-us/background/). Even for expert taxonomists, it is not trivial to identify pre-imaginal stages or a mass of adult individuals of closely related species in Drosophilidae. In Colocasiomyia, it is known in a number of cases, including that given by the present study, that closely related species cohabit on the same host plant. To study mechanisms for cohabitation in such systems, DNA barcoding approaches should be very useful in species identification, especially for pre-imaginal stages. However, the "barcoding gap" between intra- and interspecific genetic distances based on COI sequences is too flimsy to validate the objective, distance-based species delimitation in the gigantea group. Similarly, in some previous studies, distance-based DNA barcoding based on COI sequences did not work well for species delimitation due to the overlapping of inter- and intraspecific distances (see DeSalle et al. 2005, and references therein). DeSalle et al. (2005) thus proposed a character-based approach to overcome the shortcomings of distance-based species identification (e.g., lacking of objective distance criterion). In the present study, both morphological characters and DNA barcodes were incorporated into species delineation in the gigantea group: some nucleotide sites with fixed status in the COI sequences were recognized as "pure" diagnostics for each species of the gigantea group. However, the effectiveness of such diagnoses still need to be verified by sampling more sequences from each species and strengthened by sequencing more genes.

Unlike the other *Colocasiomyia* species, all the species of the *gigantea* group breed on (*C. longivalva* at least visits) inflorescences/infructescences of the subfamily Monsteroideae (Araceae). All of the host plants are hemiepiphytic, bisexual-flowered climbers belonging to the *Rhaphidophora* clade (Cusimano et al. 2011, Nauheimer et al. 2012) in the Monsteroideae. *Colocasiomyia scindapsae* and *C. gigantea* monopolize

their host plants, *Scindapsus coriaceus* and *Epipremnum pinnatum* in Sabah and Java, respectively (Fartyal et al. 2013). In this study, the five Chinese species of the *gigantea* group were found sharing the same host plant, *R. decursiva*. Moreover, one of them, *C. rhaphidophorae*, utilizes also *R. hookeri* as a host plant in Xishuangbanna, southern Yunnan (Fartyal et al. 2013).

The monsteroid plants as hosts of the *gigantea* group are quite different in the structure of spadix and the fruiting process from the Aroideae known as hosts of other *Colocasiomyia* species groups. Fartyal et al. (2013) have revealed that, in single-host/single-user systems, the *gigantea*-group species have evolved peculiar lifecycles, ecological traits and morphological features adaptive to characteristics of the monsteroid host plants. According to our preliminary observations, similar adaptations are also seen in the species cohabiting on *R. decursiva* (Li et al. unpubl. data). The discovery of such a system in which multiple species of the *gigantea* group cohabit provides an opportunity to study parallel evolution of breeding habits to achieve the cohabitation: it is known, in the *cristata* species group, that breeding habits vary according to the number and combination of cohabiting species (Carson and Okada 1980, Toda and Okada 1983, Honda-Yafuso 1983, Yafuso 1994, Takenaka 2006, Takenaka et al. 2006, Takano et al. 2012).

So far, our knowledge about the biogeography of the *gigantea* group is apparently very limited with respect to the known geographical distribution of respective host plants. Colocasiomyia scindapsae has been recorded only from Mt. Kinabalu (Sabah), C. gigantea from Bogor (West Java) and Solomon Is., and the others from Yunnan, China. The ranges of their host plant species are wider: S. coriaceus is distributed in Indonesia and Malaysia (http://gwannon.com/species/Scindapsus-coriaceus), R. hookeri in China, Myanmar and Vietnam (http://www.gwannon.com/species/Rhaphidophora-hookeri), R. decursiva in Himalaya, India and Indonesia (http://en.hortipedia.com/ wiki/Raphidophora\_decursiva), and E. pinnatum being native to New Guinea, the Malay Archipelago and the Pacific Islands (http://en.hortipedia.com/wiki/Epipremnum\_pinnatum) but currently distributed almost all over the Oriental and Australasian Region (Li 1996). In addition, the *Rhaphidophora* clade consists of approximately 170 species (Tam et al. 2004), which may include many potential hosts for Colocasiomyia flies. It is very likely, in the future, that more species of the gigantea group will be discovered from different host plants, and that more distribution and host-plant records will be brought from a wider geographic range. Such information is badly needed to explore the evolution of pollination mutualisms between flies of the gigantea group and their monsteroid host plants.

# **Acknowledgements**

We thank Mr RS Lin and GM Li (the Baihualing Village) for their help in the field work, and Mr R He (Research Institute of Resources Insects, the Chinese Academy of Forestry) for his help in SEM photographing. This work was supported by the National Science Foundation of China (No. 31160429), the fund of the Ministry

of Science and Technology of China (No. 2011FY120200 and 2012FY110800), and Grants-in-Aid for Scientific Research from Japan Society for the Promotion of Science (Nos 21570085 and 24370033).

#### References

- Carson HL, Okada T (1980) Drosophilidae associated with flowers in Papua New Guinea. I. *Colocasia esculenta*. Kontyû 48: 15–29.
- Cusimano N, Bogner J, Maya SJ, Boyce PC, Wong SY, Hesse M, Hetterscheid WLA, Keating R, French JC (2011) Relationships within the Araceae: comparison of morphological patterns with molecular phylogenies. American Journal of Botany 98: 654–668. doi: 10.3732/ajb.1000158
- DeSalle R, Egan MG, Siddall M (2005) The unholy trinity: taxonomy, species delimitation and DNA barcoding. Philosophical Transactions of the Royal Society B 360: 1905–1916. doi: 10.1098/rstb.2005.1722
- Fartyal RS, Gao JJ, Toda MJ, Hu YG, Takano KT, Suwito A, Katoh T, Takigahira T, Yin JT (2013) Colocasiomyia (Diptera: Drosophilidae) revised phylogenetically, with a new species group having peculiar lifecycles on monsteroid (Araceae) host plants. Systematic Entomology 38: 763–782. doi: 10.1111/syen.12027
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294–299.
- Hebert PDN, Cywinska A, Ball SL, de Waard JR (2003) Biological identifications through DNA barcodes. Proceedings of the Royal Society of London, Series B 270: 313–321. doi: 10.1098/rspb.2002.2218
- Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. Proceedings of the National Academy of Sciences of the United States of America 101: 14812–14817. doi: 10.1073/pnas.0406166101
- Honda-Yafuso M (1983) Interspecific relationship between synhospitalic *Drosophilella* species (Diptera, Drosophilidae) inhabiting *Alocasia odora* on Okinawa Is., Japan. Kontyû 51: 520–527.
- Li H (1996) The ecological phytogeography and origin of the family Araceae. Acta Botanica Yunnanica 18: 14–42.
- MaAlpine JF (1981) Morphology and terminology: adults. In: MaAlpine JF, Peterson BV, Shewell GE, Teskey HJ, Vockeroth JR, Wood DM (Eds) Manual of Nearctic Diptera, Vol 1. Biosystematics Research Institute, Ottawa, 9–63.
- Meyer CP, Paulay G (2005) DNA barcoding: error rates based on comprehensive sampling. PLoS Biology 3: e422. doi: 10.1371/journal.pbio.0030422
- Nauheimer L, Metzler D, Renner SS (2012) Global history of the ancient monocot family Araceae inferred with models accounting for past continental positions and previous ranges based on fossils. New Phytologist 195: 938–950. doi: 10.1111/j.1469-8137.2012.04220.x

- Okada T (1990) Subdivision of the genus *Colocasiomyia* de Meijere (Diptera, Drosophilidae) with descriptions of two new species from Sulawesi and notes on color adaptation of synhospitalic species. Proceedings of the Japanese Society of Systematic Zoology 42: 66–72.
- Sarkar IN, Thornton JW, Planet PJ, Figurski DH, Schierwater B, DeSalle R (2002) An automated phylogenetic key for classifying homeoboxes. Molecular Phylogenetics and Evolution 24: 388–399. doi: 10.1016/S1055-7903(02)00259-2
- Sultana F, Hu YG, Toda MJ, Takenaka K, Yafuso M (2006) Phylogeny and classification of *Colocasiomyia* (Diptera, Drosophilidae), and its evolution of pollination mutualism with aroid plants. Systematic Entomology 31: 684–702.
- Sultana F, Toda MJ, Yafuso M, Lakim MB, Mohamed MB, Cuong NM (2002) A new species-group of the genus *Colocasiomyia* de Meijere (Diptera: Drosophilidae), with descriptions of two new species from eastern Malaysia and Vietnam. Entomological Science 5: 305–315. doi: 10.1111/j.1365-3113.2006.00344.x
- Takano KT, Repin R, Mohamed MB, Toda MJ (2012) Pollination mutualism between *Alocasia macrorrhizos* (Araceae) and two taxonomically undescribed *Colocasiomyia* species (Diptera: Drosophilidae) in Sabah, Borneo. Plant Biology 14: 555–564. doi: 10.1111/j.1438-8677.2011.00541.x
- Takenaka K (2006) Reproductive ecology of the genus *Colocasiomyia* (Diptera: Drosophilidae) and pollination mutualism with Araceae plants. PhD thesis, Hokkaido University, Sapporo, Japan.
- Takenaka K, Yin JT, Wen SY, Toda MJ (2006) Pollination mutualism between a new species of the genus *Colocasiomyia* de Meijere (Diptera: Drosophilidae) and *Steudnera colocasiifolia* (Araceae) in Yunnan, China. Entomological Science 9: 79–91. doi: 10.1111/j.1479-8298.2006.00156.x
- Tam SM, Boyce PC, Upson TM, Barabé D, Brunear A, Forest F, Parker JS (2004) Intergeneric and infrafamilial phylogeny of subfamily Monsteroideae (Araceae) revised by chloroplast *trnL-F* sequences. American Journal of Botany 91: 490–498. doi: 10.3732/ajb.91.3.490
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Molecular Biology and Evolution 28: 2731–2739. doi: 10.1093/molbev/msr121
- Toda MJ, Okada T (1983) Ecological studies of floricolous *Drosophilella* in Burma with descriptions of three new species from Burma and Philippines (Diptera, Drosophilidae). Kontyû 51: 169–184.
- Yafuso M (1994) Life history traits related to resource partitioning between synhospitalic species of *Colocasiomyia* (Diptera, Drosophilidae) breeding in inflorescences of *Alocasia odora* (Araceae). Ecological Entomology 19: 65–73. doi: 10.1111/j.1365-2311.1994. tb00391.x
- Zhang WX, Toda MJ (1992) A new species-subgroup of the *Drosophila immigrans* species-group (Diptera, Drosophilidae), with description of two new species from China and revision of taxonomic terminology. Japanese Journal of Entomology 60: 839–850.